allows us to look at finer details such as DNA slipping and the effect of DNA sequences on unwinding rates.
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387-Pos Board B187
Displaced Strand Regulation of Facxpd Helicase Activity
Zhi Qi, Robert A. Pugh, Maria Spies, Yann R. Chemla.
FacXPD is the archaeal homolog of yeast Rad3 and human xeroderma pigmentosum group D protein (XPD) helicase from Ferroplasma acidarmanus. This enzyme serves as a model for understanding the molecular mechanism of human Superfamily 2 helicase XPD involved in both transcription initiation and nucleotide excision repair, and for the related 5'-3' helicases FancJ, Rtel and ChlR1 important for maintaining genomic integrity and DNA repair. We developed a single-molecule, high-resolution optical tweezers assay to decipher the mechanism by which a single XPD helicase unwinds dsDNA while translocating in the 5'-3' direction. This assay monitors the unwinding of an 89-bp DNA hairpin substrate with single base pair resolution. Our substrate design allows us to control the length of a poly-dT ssDNA ''translocation'' strand (the strand to which XPD binds and along which it translocates), and a ''displaced'' strand (the strand displaced upon unwinding the duplex), located at the 5' and 3' tails of the hairpin, respectively. We found that the displaced strand interacts with XPD and that this interaction controls the helicase activity. When the 3' tail of the substrate hairpin is substituted for dsDNA, a single XPD molecule displays repetitive ''non-processive'' bursts of substrate unwinding in which only ~10-bp of the hairpin is unwound at a time. However, in the presence of a ssDNA displaced strand (of length ranging from 3 to 10 nt), we observe two types of activity: the same 10-bp non-processive mode as above, and also a ''processive'' mode in which the entire 89-bp hairpin is unwound. These data suggest two different binding modes for XPD resulting in non-processive or processive unwinding which are regulated by its interaction with the displaced strand. We propose a model for how the domains of XPD bind to its DNA substrate in these two modes.

388-Pos Board B188
Investigating Hexameric Helicases: Single-Molecule Studies of DnaB and T4 Gp41
Noah Ribeck, John Berezney, Omar A. Saleh.
Hexameric, ring-shaped motor proteins serve as replicative helicases in many systems. They function by encircling and translocating along ssDNA, denaturing dsDNA in advance of its motion by sterically occluding the complementary strand to the outside of the ring. We investigate the helicase activity of two such motors using single-molecule measurements with magnetic tweezers. First, we measure the activity of the *E. coli* helicase DnaB complexed with the tau subunit of the Pol III holoenzyme. Tau is known from bulk measurements to stimulate DnaB activity (Kim et al., *Cell*, 1996); we investigate the means of this stimulation. Second, we measure helicase activity of the T4 phage helicase gp41 in multiple tethered DNA geometries. Previous work on DnaB showed a dependence of helicase activity on DNA geometry (Ribeck et al., *Biophys. J.*, 2010); here, we test gp41 for similar behavior to see whether it is a common characteristic of hexameric helicases.

389-Pos Board B189
The Escherichia Coli PriA Helicase Specifically Recognizes Gapped DNA Substrates
Michal R. Szymanski, Maria J. Jezewska, Wlodek Bujalowski.
The primosome is a multi-protein-DNA complex that catalyzes the priming of the DNA during the replication process. In Escherichia coli, PriA helicase plays a fundamental role in the initiation of the ordered assembly of the primosome. PriA is involved in recombination and repair processes being a major factor that initiates the restart of the stalled replication fork at the damaged DNA sites. This happens, presumably, through the recognition of the damaged DNA site structure though the nature of this recognition process is unknown. Here we present quantitative studies of the ssDNA gap recognition by the PriA helicase and the effect of the nucleotide cofactors on the recognition process. The data indicate a surprisingly low minimum total site size of the enzyme in the gap complex, which is ~7 nucleotides or bp as compared with the site size of ~20 nucleotides of the enzyme-ssDNA complex. The low stoichiometry indicates that the helicase exclusively engages the strong DNA-binding subsite in the gap complex and assumes a very different orientation, compared with the complex with the ssDNA. The PriA helicase binds the ssDNA gaps with 4-5 nucleotides with the highest affinity without engaging in cooperative interactions with the enzyme molecules associated with the surrounding dsDNA. Binding of ADP to strong and weak nucleotide-binding sites of the enzyme profoundly affects the affinity and stoichiometry of the helicase-gapped DNA complex. These observations are of fundamental importance for understanding of the enzyme mechanisms in both replication and recombination processes.

390-Pos Board B190
Direct Observation of Replicative Helicase DnaC by Single-Molecule Tethered Particle Motion (TPM) Experiments
Shih-Wei Liu, Yu-Hua Lo, Hung-Wen Li, Chwan-Deng Hsiao.
DNA helicases play essential roles in DNA replication, repair and recombination. *Geobacillus kaustophilusGk*DnaC is a hexameric helicase that unwinds DNA in a 5' to 3' direction during DNA replication. In this study, we developed a single-molecule tethered particle motion (TPM) experiment to monitor individual *Gk*DnaC helicases unwinding fork-like 90 bp DNA molecules in real-time. The increasing Brownian motion of DNA tether correlates with ssDNA product unwound by *Gk*DnaC helicase with strong ATP dependence, allowing the determination of unwinding rates at the single-molecule level. The average unwinding rate of *Gk*DnaC alone is determined to be 3.58 +/- 0.89 (bp/s) at 5 mM ATP. When *Gk*DnaC is complexed with primase (*Gk*DnaG), the unwinding rate showed a ~ two-fold increase, consistent with the increased unwinding product by *Gk*DnaC/DnaG complex in the ensemble gel-shift experiments. However, when *Gk*DnaC is associated with a helicase loading factor, *Gk*DnaI, the complex revealed no DNA unwinding activities. These results suggest that the assembly of a stable pre-primosome efficiently speeds up the unwinding process. We also investigated the effect of *Gk*DnaC-ssDNA interactions on the unwinding rates. A R332A mutation, in which an arginine-mediated DNA binding through salt-bridging with phosphate backbones of ssDNA is removed, accelerates the unwinding rate 2~3-folds. Mutations on other residues not related to DNA binding do not alter the unwinding rates. These single-molecule measurements suggest that enzyme translocation on the ssDNA plays an imperative part in the unwinding process.

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Position-Specific DNA Base Pair 'BREATHING' at the Replication Fork Junction Regulates Helicase Access
Davis Jose, Steven E. Weitzel, Peter H. von Hippel.
We previously used near UV circular dichroism and fluorescence spectroscopy of DNA base analogues to measure position-specific DNA 'breathing' fluctuations at model replication fork DNA constructs (Jose et al., PNAS, 106, 4231, 2009). Building on that study we have now site-specifically inserted 2-aminopurine bases into such constructs on both sides of the fork junction as spectroscopic probes to monitor specific interactions with an unwinding helicase in 'real time'. The tight-binding, stable and highly active primosome assembly of the bacteriophage T4 DNA replication system was used as the helicase, and was formed into an active unwinding initiation complex by assembling T4 helicase and primase subunits in a 6:1 subunit ratio in the presence of the non-hydrolyzable GTP-analogue, GTPγS. (The addition of hydrolyzable GTP to this initiation complex results in complete unwinding of the model replication fork.) The binding of this helicase initiation complex at the replication fork, primarily via backbone contacts with the leading strand, traps the first breathing base pair of the fork in an open conformation. The other base and base pair positions were examined to map the interactions of the bound helicase on both sides of the fork. These results suggest that this replication helicase unwinds DNA by a primarily 'passive' mechanism, with unwinding depending on DNA breathing.

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Ultrafast Fluorescence Decay Profiles Reveal Differential Unstacking of 2-Aminopurine Residues Positioned Within Binding Subsites of a Single-Stranded DNA Binding Protein
Hieu-Ngoc Nguyen, Liang Zhao, Carla W. Gray, Donald M. Gray, Tianbing Xia.
DNAs bound by proteins are kinetically dynamic and exist in multiple conformations, including conformations that differ in stacking interactions. We have utilized ultrafast time resolved fluorescence spectroscopy to investigate changes in base stacking of single-stranded DNA (ssDNA) upon binding of the dimeric gene 5 protein (g5p) of fd, f1, and M13 strains of *E. coli* bacteriophages. DNA oligomer hairpins of 44 nucleotides were designed to have two antiparallel 16-nucleotide ssDNA tails for binding of a cluster of g5p dimers. Otherwise identical oligomers each contained a single fluorescent 2-aminopurine (2AP) base. Time domain fluorescence measurements showed that the label exists in an ensemble of conformations, including stacked, partially stacked, and unstacked species that are altered upon binding of the g5p. Two oligomers had 2AP labels at different subsite locations within one of the four-nucleotide DNA-binding sites of a given g5p monomer when the oligomers were saturated with g5p. These labels showed increases of the unstacked conformation from 22-24% for the free DNAs to 47-74% for the bound DNAs, where the extent of this increase was specific for the subsite location within the DNA-binding site of the g5p monomer. Time resolved anisotropy measurements indicated that the 2AP labels were in relatively inflexible conformations within the g5p-saturated oligomers.